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Numerical Relation of Spermatozoa to Sertoli Cells.[1]

FLORENCE S. HAGUE.

[Contribution from the Zoölogical Laboratory No. 213.]

THIS study was started with the purpose of ascertaining the number of spermatozoa associated with each Sertoli cell in the human testis. However, on account of being unable to get material which was in the right condition, the problem was changed to lower mammals.[2]

The tissues used were from the testes of man, the cat, and the rat. Nothing was known as to the age or condition, healthy or otherwise, of the human material. The pieces sectioned had been preserved in formalin. Several celloidin mounts were also studied. Flemming's tri-color, Davis' cytological stain, and iron hæmatoxylin with acid fuchsin, and eosin, as counterstains, gave the best results. Delafield's hæmatoxlyn with orange G, congoroth, and bordeaux red resulted in very lightly stained sections. For fixing the rat testes Mueller's, Gilson's, Bouin's, Tellyesniczky's and Zenker's fluids were used, the last two of which were the most satisfactory. The cat testes were preserved in Tellyesniczky's and Flemming's solutions. For sections of both the rat and cat testes tri-color, iron hæmatoxylin with or without eosin, dilute Delafield's hæmatoxylin and hæmalum were used. The sections varied in thickness from 6 to 40 micra.

1. I wish to acknowledge the assistance and direction of Prof. W. J. Baumgartner and Dr. B. M. Allen in this problem.

2. The numerical relation is now being worked out in the cat. A similar study is planned for other forms.

The only statements of ratios of spermatozoa to Sertoli cells, thus far found, are those of Montgomery and Winiwarther. Both of these base their figures on the proportion—3 to 1—of spermatogonia without rods to spermatogonia with rods, or crystals. They agree that from the cells containing rods Sertoli cells develop. However, Winiwarther says that the three cells with rods develop into primary spermatocytes, while Montgomery asserts that they divide before becoming primary spermatocytes. The latter thus gets the ratio of 24 spermatozoa to one Sertoli cell, and the former the ratio of 12 spermatozoa to each Sertoli cell.

After comparatively little study of mature Sertoli cells and spermatozoa in the rat, the conclusion was reached that there could not be as many as 24 spermatozoa to each Sertoli cell. Then the theory that there are 12 spermatozoa to each Sertoli cell was assumed, but this too had to be abandoned, since the counts showed smaller numbers.

In the human material available the spermatozoa have nearly all been expelled. Accordingly, distinct Sertoli cells filled with spermatozoa are hard to find. Three cells were found in which there were twelve spermatozoa, a few in which there were ten or eleven, and more in which there were eight or nine spermatozoa. However, the writer has no way of telling whether or not the Sertoli cells are complete, for the sections are only 10 micra thick, with the exception of the celloidin ones. The few spermatids are not grouped together thus making counts impossible.

The rat testes were taken from two animals, and were in a condition of very active production of spermatozoa. In the rat the mature Sertoli cells are long and slender, and have the appearance of striations. The spermatocytes between the Sertoli cells are small and identical. Consequently tracing a Sertoli cell from one section to another is extremely difficult. Sections varying in thickness from 6 to 24 micra were stained lightly with Delafield's hæmatoxylin or hæmalum. The spermatozoa, however, took the stain so lightly that they can not be distinguished when packed in the cells. Sections 15 micra thick stained with iron hæmatoxlyin are so much better, that some counts were made from them, also from a section of tissue preserved in Flemming's fluid, but which was already prepared. Thinking that there were twelve spermatozoa in

each Sertoli cell, and that the cells might not always be complete, only the larger numbers were tabulated. The following are the counts:

11 Sertoli cells with 12 spermatozoa.
18 Sertoli cells with 11 spermatozoa.
20 Sertoli cells with 10 spermatozoa.

In another effort to trace Sertoli cells from one section to another, several successive cross sections of tubules of 15-micra sections were outlined, and the groups of spermatozoa were located and counted. Even though there was no success in tracing the cells, there were other results from these counts. Since Sertoli cells are about 50 micra long and 12 micra thick when fully developed, there should be in sections 15 micra thick, some complete cells, or cells with 12 spermatozoa, about equal numbers of cells with 11 and 1, with 10 and 2, with 9 and 3, with 8 and 4, with 7 and 5, and about twice as many with 6 spermatozoa, providing there are 12 spermatozoa in each Sertoli cell. But the following are the numbers in 6 sections:

14 groups of 1 each.
26 groups of 2 each.
32 groups of 3 each.
32 groups of 4 each.
57 groups of 5 each.
70 groups of 6 each.
65 groups of 7 each.
35 groups of 8 each.
13 groups of 9 each.
6 groups of 10 each.
2 groups of 11 each.
3 groups of 12 each.

Since this did not give any definite ratio, groups in eight sections 8 micra thick were counted, with the following results:

29 groups of 1 each.
24 groups of 2 each.
27 groups of 3 each.
26 groups of 4 each.
49 groups of 5 each.
44 groups of 6 each.
41 groups of 7 each.
21 groups of 8 each.
6 groups of 9 each.
3 groups of 10 each.
0 group of 11
1 group of 12

In the first of these counts the sections are thick enough so that one Sertoli cell would not be in more than two sections, but the numbers of groups of 1, 2, and 3 spermatozoa far surpassed the numbers of groups of 9, 10, and 11. From this it seems that the number can not always be as high as twelve, and seldom, if ever, higher. In the 8-micra sections, it would be possible for a Sertoli cell to be in three sections, and this would account for a larger number of small groups of spermatozoa, but hardly for as great a difference as there is. In both counts the numbers of groups of 5, 6, and 7, are far in excess of other numbers.

Short pieces of unstained tubules were teased and stained. Some pieces of tubules were stained in alum cochineal, or Delafield's hæmatoxylin, cleared in cresote and then teased or cut longitudinally and spread out. One difficulty here is being unable to determine the stage of development of the spermatozoa. It is quite seldom, too, that the Sertoli cells are sufficiently isolated to enable one to make counts of their contents. No counts were recorded, but in those cells which seem to be complete there are more often 8, 9, or 10 spermatozoa than 11 or 12.

Finally, sections 20, 25, 30, 35, and 40 micra thick were prepared and stained with iron hæmatoxylin with or without eosin, and with tricolor. The tricolor did not give as good results, though less destaining would probably have improved it. With iron hæmatoxylin, the spermatogonia, spermatocytes and spermatids are all a light brown, and the spermatozoa a dead black when mature. Thus especially with an immersion oil objective, the spermatozoa are clear and distinct. In the 20-micra sections a few Sertoli cells can be found, the spermatozoa of which are invisible at both the upper and lower plane of the section. In sections 30 micra thick a Sertoli cell with 7 or 8 spermatozoa may be in focus at the upper level of the section, may disappear, and at the lower level another cell immediately beneath the first and equally well filled may come into focus. In 40-micra sections a third cell may sometimes be seen directly above two others. Thus there can be no doubt but what complete cells are contained in these sections.

In all counts care was taken to have the complete thickness of the cell, and in practically all the entire length could also be traced. Counts were made in several stages. The clearest and easiest were those made when the spermatozoa were approaching and at the base of the cell, or at the base and begin-

ning to move outward. In both these conditions each Sertoli cell is almost invariably completely surrounded by spermatocytes. In the latter case sections of tubules with the lengths of the lumens extending across the entire field, with the low power, were used and no spermatozoa were seen in the lumens, or had even reached the inner portion of the Sertoli cells. Counts of very early stages of spermatozoa were very difficult, because they are less clearly outlined, and the Sertoli cells are thicker, more branching, and come in contact with each other about the lumen, thus making the groups of immature spermatozoa less distinct than the mature groups. The spermatids are not sufficiently grouped to give reliable accounts.

In all the following counts the microscope was carefully focused each time to see that the cell was complete. First mature spermatozoa were counted in longitudinal sections of tubules, in the lumens of which there were no spermatozoa; neither had the spermatozoa advanced to the inner portion of the Sertoli cells. Thus none can have escaped. The following groups of spermatozoa in Sertoli cells were counted in sections 30, 35, and 40 micra thick:

10 groups of 6 spermatozoa.
30 groups of 7 spermatozoa.
45 groups of 8 spermatozoa.
35 groups of 9 spermatozoa.
3 groups of 10 spermatozoa.

In sections of the same thickness groups of spermatozoa which had just reached or were approaching the bases of the Sertoli cells were counted, and the following results obtained:

9 groups of 6 spermatozoa.
26 groups of 7 spermatozoa.
24 groups of 8 spermatozoa.
16 groups of 9 spermtaozoa.
5 groups of 10 spermatozoa.
4 groups of 11 spermatozoa.

Finally very early spermatozoa were counted in sections 40 micra thick, most of which were stained with iron hæmatoxylin and counterstained with eosin. The counts of these immature spermatozoa are as follows:

10 groups of 6 spermatozoa.
23 groups of 7 spermatozoa.
27 groups of 8 spermatozoa.
25 groups of 9 spermatozoa.
10 groups of 10 spermatozoa.
2 groups of 11 spermatozoa.
1 group of 12 spermatozoa.

The total of 313 counts from sections 30, 35, and 40 micra thick are:

29	groups of	6 spermatozoa.
77	groups of	7 spermatozoa.
106	groups of	8 spermatozoa.
76	groups of	9 spermatozoa.
18	groups of	10 spermatozoa.
6	groups of	11 spermatozoa.
1	group of	12 spermatozoa.

In two cells which appeared to be whole only 5 spermatozoa could be made out, and a few times, especially among immature spermatozoa, groups were so close together as at first to appear as one, thus forming groups of more than twelve. It is possible that groups of 5 or 13 or 14 do occur, but if so, they are very rare. From these counts and the earlier ones it is evident that the number of spermatozoa to each Sertoli cell in the rat varies from 6 to 12 and is oftener 7, 8 or 9.

The rod or crystal which Montgomery and Winiwarther describe is not visible in the human material studied, but this can doubtless be accounted for in the fixation. The Sertoli cell nuclei stain darkly and are always irregularly shaped and distributed. The Sertoli cells of the cat testes on the contrary do show rods and rodlets, in the tissue preserved in Tellyesniczky's fluid as well as that preserved in Flemming's fluid. These rods are, presumably, structures similar to those of human Sertoli cells for they are long and slender, sometimes curved, and irregularly placed. They are, however, not as distinct as those of the human testes as illustrated by Montgomery and Winiwarther. It is only with very careful focusing that they can be seen at all, and a few times this focusing has made them appear to be corners or edges of the nuclear membrane. With their irregularly shaped nuclei this might indeed be possible. Winiwarther, although, he calls these structures crystals, and has discussed them at some length, has been unable to prove their crystalline properties by polarization of light and even says that he has never been able to identify with certainty these crystals of Charcot. Besides the rod or crystal, both authors describe rodlets or accessory strips (batônnets), which Montgomery says persist longer, while Winiwarther says the rods persist after the rodlets disappear. The rods have been seen after the Sertoli nuclei were well pushed away from the wall of the cat testes.

None of the mature Sertoli cells of the rat testes show either rods or rodlets. About the periphery of a section preserved in Flemming's fluid and stained with iron hæmatoxylin, there are occasional dark streaks on the Sertoli nuclei, which at first appeared to be rods. These are not visible in the lighter cells of the central part of the section. The nuclei are uniformly slightly granular and contain a large nucleolus and two or three smaller chromatic bodies, one always close to or attached to the nucleolus.

In the human testis by far the greater part of the cells seen were primary spermatocytes in various stages, but none dividing. At the periphery there were also a few cells in earlier stages, and at the lumen some spermatids and spermatozoa. Some sections of tubules of the rat testis show a row of developing primary spermatocytes at the periphery, then secondary spermatocytes, between the long Sertoli cells which contain mature spermatozoa. Other tubules show the primary spermatocytes almost mature, secondary spermatocytes, and Sertoli cells with spermatozoa, pulling away from the wall of the tubule and passing toward the lumen. At the same time there can be seen in other tubules a row of the large primary spermatocytes inclosing a mass of secondary spermatocytes, which in turn enclose the last of the escaping spermatozoa. The secondary spermatocytes divide quickly into spermatids and then first begin to form in groups. When they are seen elongated and in definite groups about the inner ends of the Sertoli cells, which have grown inward, the mature primary spermatocytes are dividing and the next generation is beginning to appear. The spermatozoa begin to form at the periphery of the lumen, but as they condense more and as the secondary spermatocytes which are being formed, push inward, the spermatozoa make their way toward the base of the Sertoli cell, often clustering close about the nucleus. Even when passing out the nucleus is frequently very close to the spermatozoa.

Montgomery in speaking of ultimate spermatogonia one quarter of which, he says, contain rods and three quarters lack rods, says: "The ratio is somewhat less than 1 to 3, which is readily explained on the ground that some of the spermatogonia with rods had already become Sertoli cells and therefore were not included in the count." It would seem, then, that Sertoli cells develop from the ultimate spermatogonial condition before the

other three cells divide and develop into the primary spermatocytes. Then, assuming that the principles of spermatogenesis in the rat are the same as those Montgomery describes for man, there ought to be Sertoli cells, perhaps not quite mature, for each generation of germ cells in the tubule. Usually three generations of germ cells—primary and secondary spermatocytes, and spermatozoa—are distinctly visible in the tubules. But only when one generation of spermatozoa and Sertoli cells are passing out can a second generation of Sertoli cells be distinguished. Where, then, are the successive quarters of ultimate spermatogonia which contain the rod, some of which develop into Sertoli cells before the other three quarters of ultimate spermatogonia can divide? It would seem that they would at least be visible by the time the other three quarters had become secondary spermatocytes.

CONCLUSIONS.

1. The Sertoli cells of the rat do not show a rod-like structure, but those of the cat do.

2. There is no definite connection between Sertoli cells and germ cells until the spermatozoa are beginning to form.

3. The number of spermatozoa to each Sertoli cell varies from 6 to 12 in the rat.

BIBLIOGRAPHY.

Debaisieux, P.

1909. Les débuts de l'ovogènse dans le Dytiscus marginalis. La Cellule 25.

Flemming, von W.

1897. Ueber die Chromosomenzahl beim Menschen. Anat. Anz. Bd. 14.

Guyer. M. F.

1910. Accessory chromosomes in man. Biol. Bull. vol. 19.

1900. Spermatogenesis of Normal and Hybrid Pigeons.

Jordan, H. E.

1912. Heterochromosomes in mammals. Proceedings of Soc. for Experimental Biol. and Med. vol 10, No. 1.

Montgomery, Thomas H.

1911. Differentiation of the Human cells of Sertoli. Biol. Bull. vol. 21.

Morris, J. T.

1909. A note on Orange G. counterstaining suggesting a useful method in the management of embryonic tissues. Anat. Rec. vol. 3.

Regaud, C.
1899. Sur la morphologie de la cellule de Sertoli, etc. C. R. Assoc. Anat. Paris.

Stephan, P.
1902. L' évolution de la cellule de Sertoli des Sélaciens. C. R. Soc. Biol. Paris 54.

Waldeyer, W.
1906. Die Geschlechtszellen. Hertwig's Handbuch der Entw. Wirbeltiere. 1 Jena.

Walker & Embleton.
1906. On the origin of the Sertoli or Foot-cells of the Testis. University of Liverpool and Royal Infirmary Cancer Research Laboratories. First Report on the Cytological Investigation of Cancer.

Watase, S.
1892. The Origin of the Sertoli's Cell. Amer. Nat. 26.

Wieman, H. L.
1913. Chromosomes in Man. Am. Journal of Anat. vol. 14, No. 4.

Wilcox, E. V.
1900. Human Spermatogenesis. Anat. Anz. 17.

Winiwarther, H. von
1912. Etudes sur la spermatogenese humain. Arch. d. Biol., Bd. 27. Observations cytologique sur les cellules interstitulles du testicle humain. Anat. Anz. Bd. 41.